MORPHOMETRIC INVESTIGATION OF STRUCTURAL CHANGES IN THE LIVER OF MICE REPEATEDLY EXPOSED TO STRESS

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The liver plays an essential role in the mechanism of adaptive changes taking place in metabolism when the body is exposed to the action of external factors. However, the structural changes taking place in the liver under these conditions have received little study.

According to previous data [3, 4], the state of acute stress in mice led to several changes in the subcellular organization of the hepatocytes, whose existence evidently reflected the formation of structural mechanisms of "urgent adaptation" [1] in the hepatic parenchyma.

The aim of this investigation was to study structural changes in the liver in mice repeatedly exposed to vibration.

EXPERIMENTAL METHOD

Male C57BL/6 mice aged 2 months and weighing 19-21 g, with free access to water and food, were used. The animals were divided into five groups, with five mice in each group. Animals of group 1 served as the control. Animals of the other three groups were exposed to vibration between 9 and 10 a.m. daily in an AVP-4P apparatus for 45 min with a frequency of 2.5 Hz. Mice of group 2 were decapitated on the 4th day, immediately after the end of vibration, mice of group 3 were decapitated on the 10th day, immediately after vibration, and mice of group 4 on the 21st day. Mice of group 5 were decapitated at the same time of day, but after 3 days of rest following daily exposure to vibration in the manner described above for 21 days.

Specimens of liver for electron microscopy were fixed in 1% OsO_4 solution in phosphate buffer and embedded in Epon; semithin sections were obtained from them, stained with toluidine blue, and used for morphometry. Square test systems [7] were used for morphometry of the liver tissues and of the ultrastructures of the hepatocytes. The results of morphometry were subjected to statistical analysis and correlation analysis. Differences between means were considered significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

Daily exposure of the animals to stress caused no significant changes in the structural organization of the liver at tissue and cell levels (Table 1). An increase in the total volume of the sinusoidal cells by 41% (in the animals of group 5) was due to their hypertrophy, because the number of cells did not differ significantly from the control (8.47 ± 0.53 and 7.66 ± 0.39 respectively, $P > 0.05$). In animals of the other groups this parameter likewise did not differ significantly from the control. The decrease in the volume of the hepatocytes by 40% and of their nuclei by 31% compared with the control in animals of group 2 was due to division of some of the cells.

Meanwhile the subcellular organization of the hepatocytes changed significantly (Table 2). The maximum of the changes was observed on the first day after exposure (group 3). The surface area of the outer membrane of the mitochondria in the hepatocytes of these mice was increased by 30% of the control, and that of the inner membrane by 61%, the surface area of membranes of the rough endoplasmic reticulum was increased by 49%, and of lysosomal structures by 100% (Table 2). The total concentration of membranes (in μ^2/μ^3 of cytoplasm) of the hepatocyte organoids studied (Table 2) at this time exceeded that in the control by 51% (Fig. 1). This parameter was increased by a lesser degree (not more than 30%) in hepatocytes of the animals

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TABLE 1. Results of Morphometry of the Liver of C57BL/6 Mice according to Data of Light-Optical Microscopy (M ± m)

Parameter	Group of animals				
	1	2	3	4	5
Volume of parenchyma (V_V), %	86,1±1,13	84,8±0,83	88,8±1,18	87,9±1,16	81,6±1,21*
Total volume of sinusoidal cells (V_V), %	9,2±0,61	8,7±0,56	8,8±0,69	9,7±0,71	12,9±0,86*
Volume of sinusoids, μ^3	5,1±0,60	7,0±0,66	2,4±0,43*	2,6±0,40	5,6±0,67
Volume of hepatocyte nuclei, μ^3	330,6±9,30	252,2±2,73*	356,6±3,43	378,9±3,72*	321,3±3,08
Volume of hepatocyte cytoplasm, μ^3	3802±319	2706±176*	3103±303	4490±389	3360±245
Volume of hepatocytes, M^3	4132,6±319	2958,2±176*	3459,6±303	4869,9±389	3681,3±245

Legend. V_V) Relative volume. Here and in Table 2, asterisk indicates significant difference from control.

TABLE 2. Results of Morphometry of Subcellular Structures of Hepatocytes of C57BL/6 mice (M ± m)

Parameter	Group of animals				
	1	2	3	4	5
V_V of mitochondria (outer membrane)	16,7±0,87	24,0±0,98*	20,8±1,04*	20,8±0,94*	21,2±0,76*
S_V of mitochondria (inner membrane)	0,95±0,039	1,32±0,045*	1,24±0,052*	1,21±0,044*	1,16±0,035*
S_V of mitochondria (inner membrane)	2,65±0,164	3,21±0,131*	4,25±0,206*	3,56±0,175*	3,68±0,168*
V_V of lysosomal structures	0,65±0,083	2,53±0,172*	2,11±0,223*	1,31±0,142*	0,57±0,008
S_V of lysosomal structures (membrane)	0,10±0,013	0,21±0,020*	0,21±0,024*	0,17±0,018*	0,08±0,011
V_V of peroxisomes	1,18±0,112	0,97±0,082	1,19±0,121	1,10±0,132	1,37±0,135
S_V of peroxisomes (membrane)	0,13±0,013	0,12±0,009	0,13±0,013	0,11±0,014	0,13±0,012
V_V of rough endoplasmic reticulum (membrane)	10,3±1,12	10,2±0,54	12,6±0,73	11,6±0,67	9,5±0,57
S_V of rough endoplasmic reticulum (membrane)	2,61±0,194	3,22±0,130*	3,90±0,183*	3,35±0,180*	3,23±0,181*
V_V of lipid inclusions	0,82±0,240	—	0,15±0,071*	0,15±0,070*	—

Legend. V_V) Bulk density (in % of volume of cytoplasm); S_V) surface density (in μ^2/μ^3 volume of cytoplasm).

of groups 4 and 5. The period of maximal increase in concentration of the membranes was preceded by an increase (by 62%) in the number of free ribosomes, maintaining a higher level of regeneration, and it was only after this (group 3) that the number of attached ribosomes, responsible for synthesis "for export", increased (Fig. 1a, b). Changes observed in the ultrastructures of the hepatocytes in mice of groups 2-4 were evidently adaptive in character, for their result was an increase in the "physiological power" [1] of the system due to an increase in the weight of its elements in response to the increased functional loads on the organ.

Besides the evident predominance of anabolic processes in hepatocytes of the mice of groups 2-4, changes in the glycogen content and also qualitative and quantitative changes in the lysosome population in connection with the duration of exposure to vibration also merits attention (Fig. 2; Table 2). For instance the glycogen content in the autophagic vacuoles in the animals' hepatocytes was characterized by strong negative correlation ($r = -0.89 \pm 0.263$). The increase in the total volume of lysosomal structures in hepatocytes of the mice of groups 2-4 was due to a considerable increase in the number of autophagic vacuoles: cytosegresomes, autophagolysosomes at a later stage of degradation of the contents, and residual bodies (Fig. 2). Particles of the last two types predominated (Fig. 3). For example, in hepatocytes of the mice of group 2 the total volume of autophagic vacuoles was 47 times greater than that in the control (Fig. 2). All the cytoplasmic structures of the hepatocytes were observed as contents of the autophagosomes. This case was evidently the manifestation of the "reconstructive function" [2] of the lysosomes which, according to the hypothesis put forward by the authors cited, is realized under extremal conditions when there is a deficiency of energy-yielding substrates and of plastic material in the cells. It is suggested that products of lysosomal hydrolysis can be used in gluconeogenesis as an additional source of energy. This way of compensating the deficiency of energy sources is perhaps realistic for the situation under discussion. For instance, according to data in the literature [7], the rate of

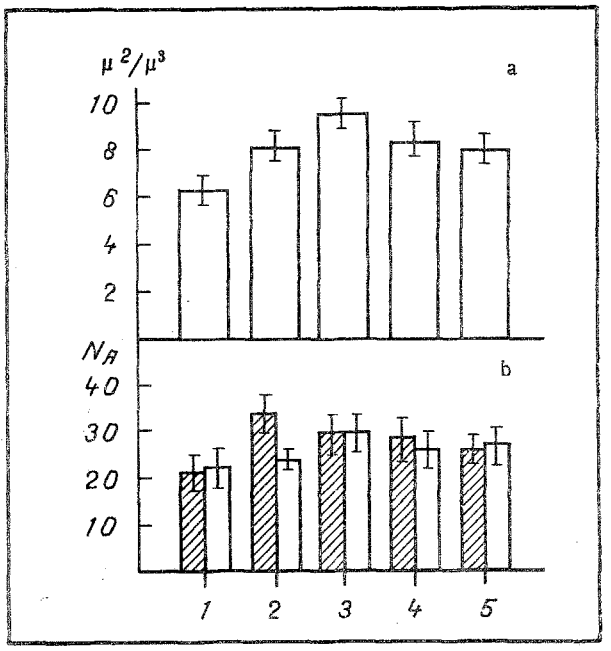

Fig. 1. Results of morphometry of mouse hepatocytes: a) total surface area of membranes of cytoplasmic structures: outer and inner membranes of mitochondira, of rough endoplasmic reticulum, peroxisomes, and lysosomal structures (in μ^2/μ^3 cytoplasm); b) number of ribosomes in 1 μ^2 area of section through cytoplasm: shaded columns — free ribosomes, unshaded columns — attached ribosomes. Abscissa, groups of animals.

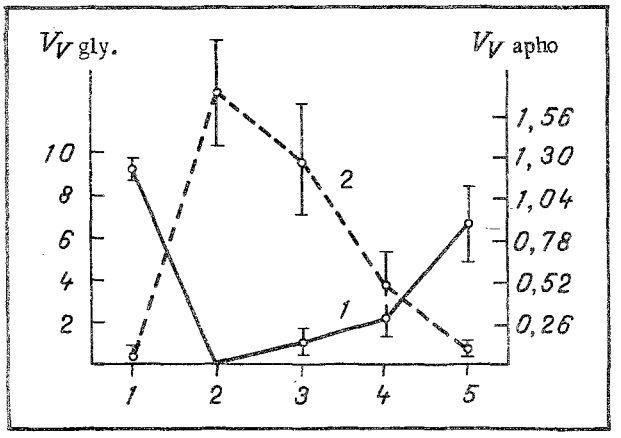

Fig. 2. Results of morphometry of glycogen and of autophagic vacuoles in mouse hepatocytes: 1) volume of glycogen (in % of volume of cytoplasm); 2) total volume of autophagic vacuoles (in % of volume of cytoplasm). Abscissa, groups of animals.

degradation of structures isolated in autophagolysosomes is quite high (the maximal half-period is 18 min). However, this way of obtaining sources of energy has a high "structural" [1] and is characteristic of processes of "urgent adaptation" [1], which were observed in mouse hepatocytes under conditions of acute stress together with a decrease in surface area of several structures, ribosomes, and glycogen [3, 4]. In autophagy induced by glucagon in

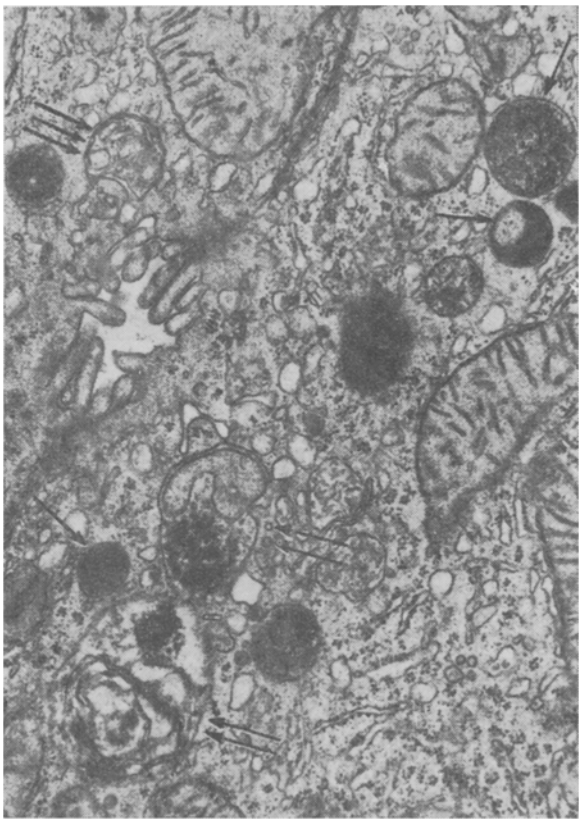

Fig. 3. Structural and functional sites of lysosomal structures in hepatocytes of animals of group 2. Arrow — lysosomes, two arrows — autophagolysosomes, three arrows — cytosegresomes. 26,000 ×.

lysosomal structures changes similar to those described in [5] took place in the content of glycogen and autophagic vacuoles. On the basis of this work and of data obtained in the present investigation it can be tentatively suggested that parallel intensification of regenerative processes in the hepatocytes and of autophagocytosis was adaptive in character and was determined by the leading role of the liver in the mechanism of adaptive changes in various metabolic processes and, in particular, in maintenance of a homeostatic level of the blood glucose concentration. During adaptation of mice to the extremal factor, the need for an "expensive" mechanism of adaptation no longer existed. For instance, despite continuation of exposure to stress, glycogen was found in the liver on the 10th day, and by the 21st day its content had increased, whereas the content of autophagic vacuoles was sharply reduced (Fig. 2). After discontinuation of the stimulus, the volume of the lysosomal structures and autophagic vacuoles in animals of group 5 returned after 3 days to values close to the control. The concentration of cell membranes under these cirucmstances was 29% higher than in the control, evidence of the formation of a "systemic structural trace" of adaptation [1]. Hyperplasia of the ultrastructures in this case, incidentally, was not accompanied by hypertrophy of the hepatocytes, as was described previously. For instance, after a single exposure to vibration after 3 days of rest the volume of the hepatocytes increased by 25% [6] whereas 3 days after administration of glucagon it rose by 38% [5].

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NEOCORTICAL ULTRASTRUCTURE DURING REHABILITATION AFTER LONG-TERM PROTEIN-CALORIC DEFICIENCY

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The principal method of treatment of patients suffering from protein-caloric deficiency in contemporary medical practice is still food rehabilitation; as a rule, however, this does not lead to complete recovery of the morphology and functions of the individual concerned [11]. Attempts to utilize hormones and, in particular, thyroid hormone, pituitary growth hormone, and adrenocortical hormones, for rehabilitation have been described [2, 13]. However, their use has not achieved widespread popularity. Previous investigations have shown that a combination of dietary rehabilitation with the addition of carnitine to the diet goes a long way toward restoring the tissue-structural changes arising in the brain after protein-caloric deficiency [8].

In the investigation described below the possibilities of dietary rehabilitation involving the use of a balanced synthetic diet and a diet with the addition of carnitine, in order to reverse the changes caused by protein-caloric deficiency, were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 34 CBA mice which received an experimental diet containing 5% of casein and a control diet with 10% of casein from the 10th through the 40th day of life [5]. Six experimental and six control animals were killed immediately after the experiment, eight experimental mice received a balanced synthetic diet for 1 month after underfeeding (dietary rehabilitation), and seven mice, in addition to a balanced diet, also were given carnitine in a dose of 833 mg/kg of food, corresponding to a dose of 0.5 mg carnitine/g body weight [6]. Seven animals receiving a balanced diet from the 10th through the 70th days of life served as the control. The carnitine was provided by the Laboratory of Pharmacology (Head, Dr. Med. Sci. V. M. Avakumov), "Vitaminy" Research-Production Combine.

Material for electron-microscopic investigation was processed by the method described previously [8].

EXPERIMENTAL RESULTS

Several changes of destructive and compensatory character were described in previous publications describing a study of the effect of malnutrition on brain ultrastructure, a series of changes of destructive and compensatory nature was described [8, 9, 12]. For instance, a decrease in the number of ribosomes and tubules of the rough endoplasmic reticulum was found in the cytoplasm of neurons of layer V of the neocortex in mice kept on a low protein diet from the 10th through the 40th day of life. Widening of the cisterns of the lamellar complex and an increase in the number of vesicles surrounding them took place. Many mitochondria had a very pale matrix and their cristae were partly destroyed (Fig. 1). Meanwhile in the perikaryon of the neurons of underfed animals many large secondary lysosomes, from 1 to 1.5 μ in diameter, and also lipofuscin granules and vacuoles appeared. The most dramatic changes in neuropil ultrastructure were found in the structure of synapses on spinous processes of the dendrites. The width of the synaptic spaces and of the postsynaptic condensations of the membranes was reduced. The spinous apparatus was substantially modified,

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